

Fig. 1. is a table showing the emission wavelength maximum (nm) of aequorin mutants with coelenterazine analogues.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S (Aeq3)	Aequorin Mutant S Ser5Cys (Aeq5)	Aequorin Mutant S Tyr132Ile
CTZ i	472	491	491	487
CTZ ip	472	470	454	453
CTZ h	472	476	471	471
CTZ hcp	472	476	448	465
CTZ cp	472	470	456	457
CTZ fcp	472	466	471	471
CTZ f	472	490	473	471
CTZ n	472	487		
CTZ native	472	474	471	471

* All values, except wild type aequorin, were calculated from the average of 3 trials (wild type with 2). All mutants were, except Aeq5 purified to >95% purity. CTZ analogues diluted to 100 micrograms/ml methanol.

FIG. 2
Emission Spectra of Aeq3 and Native Aeq With CTZ i, hcp

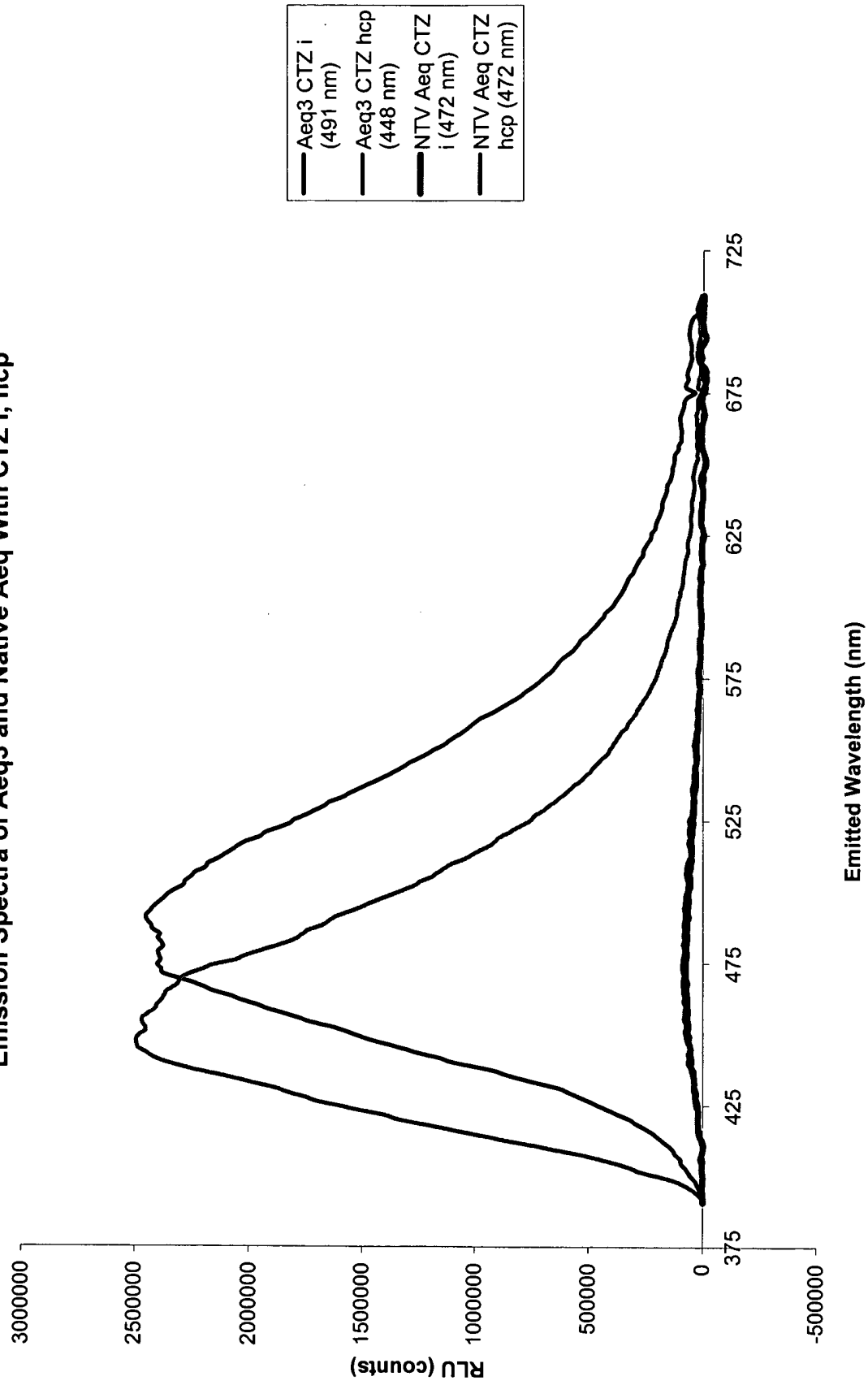


FIG. 3
Aequorin 3 With Coelenterazine (CTZ) Analogues

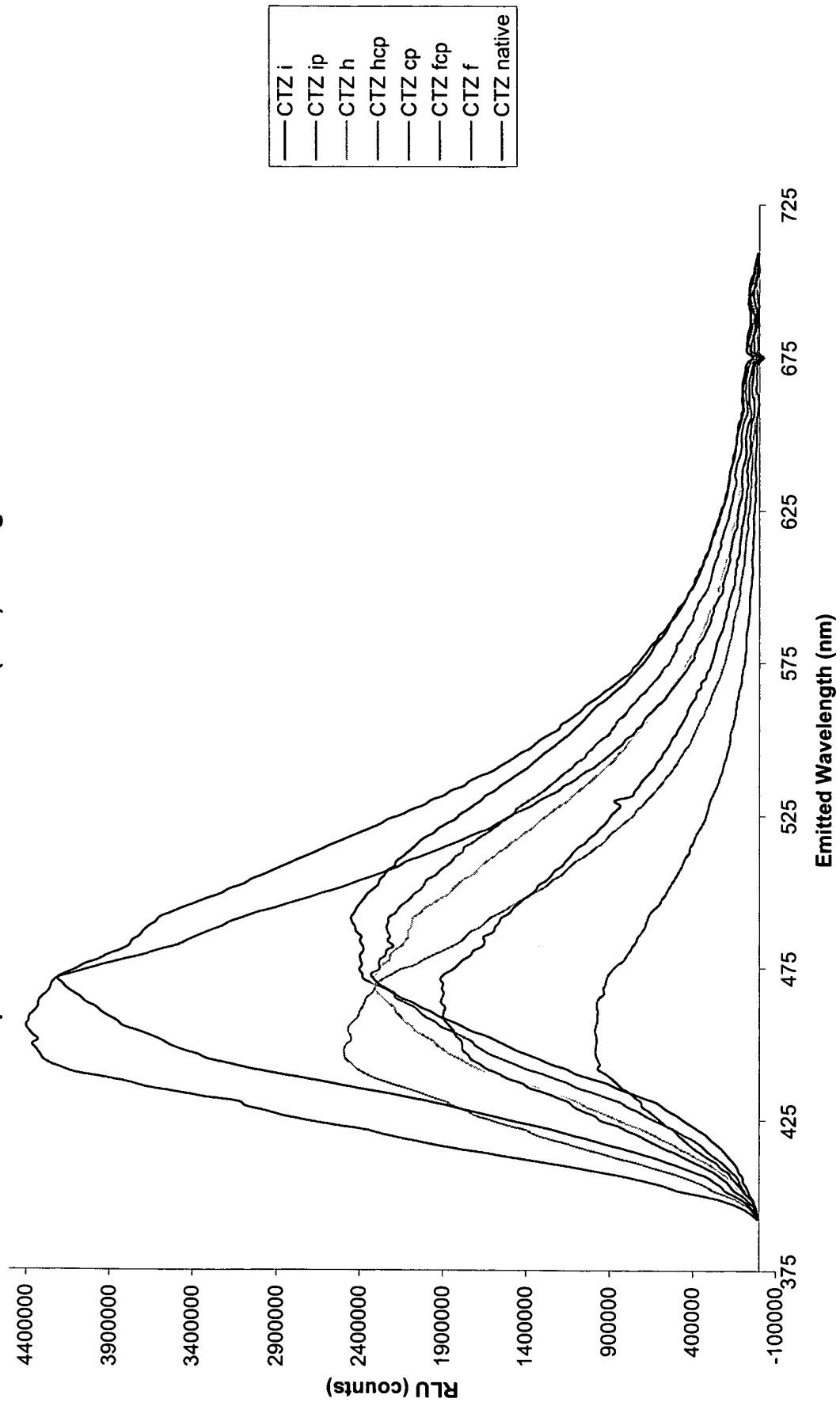
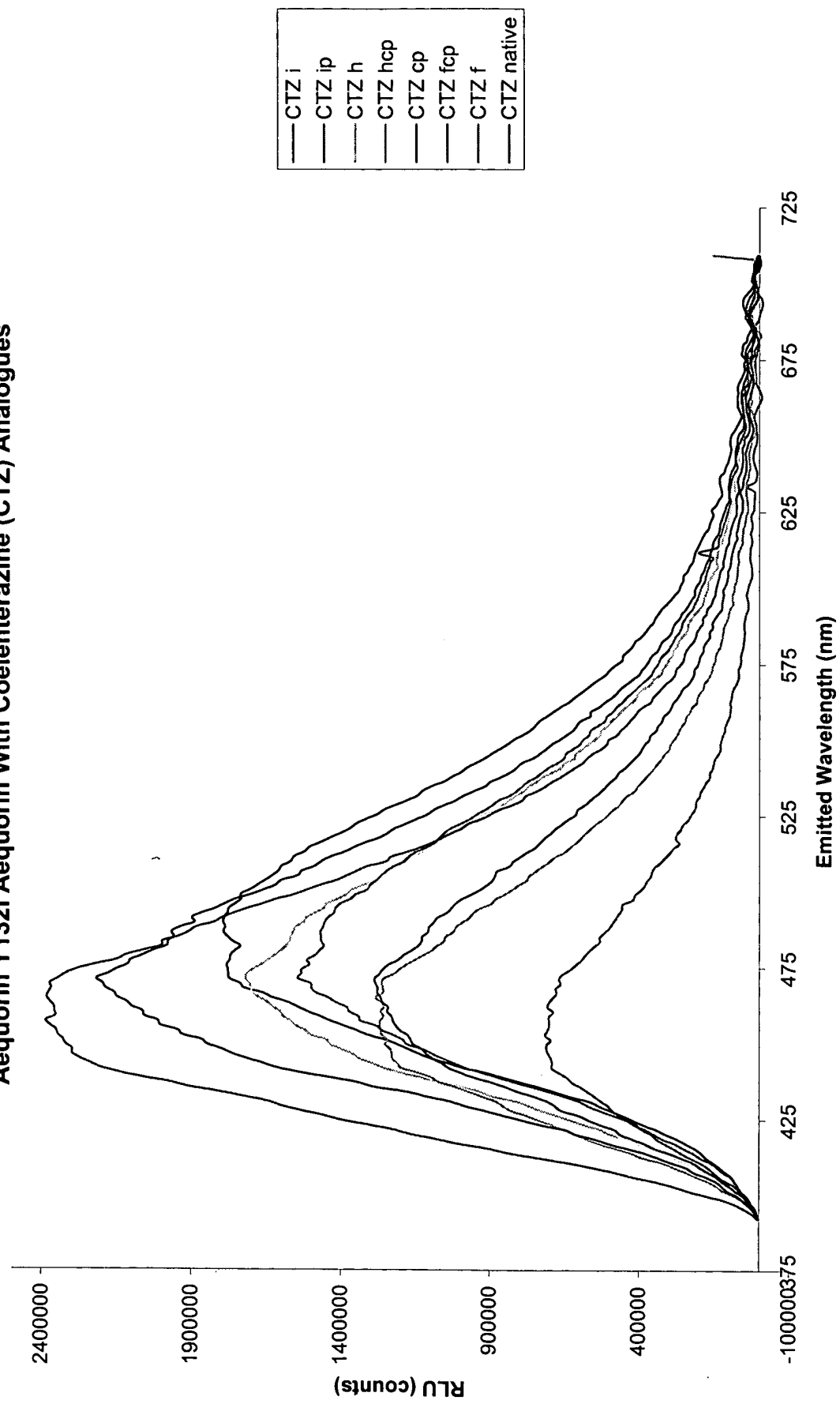


FIG. 4
Crude Aequorin 5 (Average of 2 Trials) With Coelenterazine (CTZ) Analogues



FIG. 5

Aequorin Y132I Aequorin With Coelenterazine (CTZ) Analogues



Native Aequorin with Coelenterazine (CTZ) Analogues

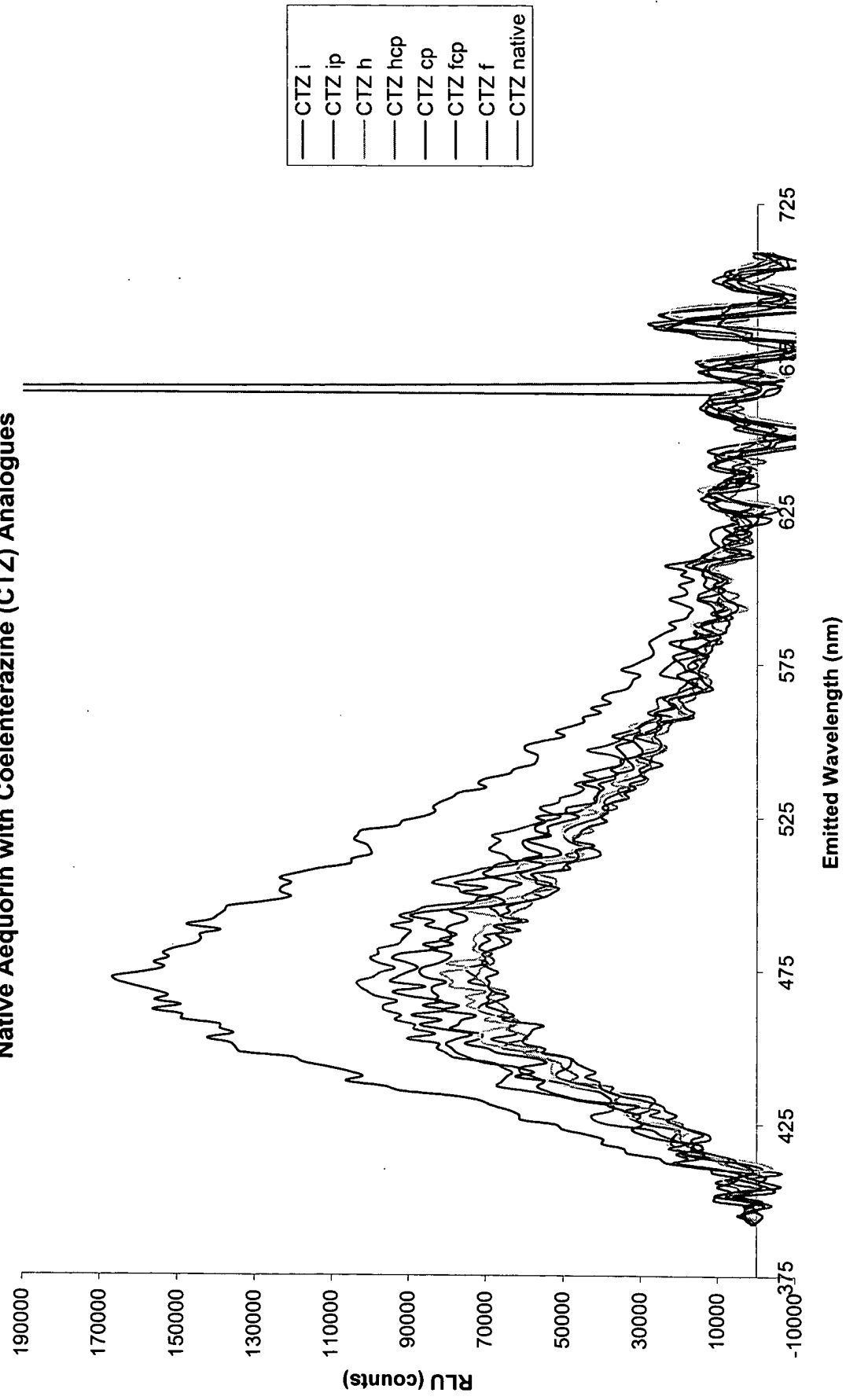


Fig. 7. is a table showing the emission wavelength maximum (nm) of aequorin mutant Mutant S Y132I, Mutant S having a 3-fluoro-l-tyrosine aequorin or a 5-fluoro-l-tyrosine non-natural amino acid in position 132 in conjunction with coelenterazine analogues CTZ i, ip, n, h, hcp, cp, fcp, f and native CTZ.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S Tyr132Ile	Aequorin Mutant S Tyr132 3-fluoro-l-tyr	Aequorin Mutant S Tyr132 5-fluoro-l-trp
CTZ i	472	491	511	495
CTZ ip	472	452	471	
CTZ n	472	491	500	
CTZ h	472	472	498	471
CTZ hcp	472	452	471	468
CTZ cp	472	457	471	471
CTZ fcp	472	463	471	
CTZ f	472	472	500	497
CTZ native	472	471	495	472

Figure 8
Spectra of 3-Fluoro-L-Tyrosine

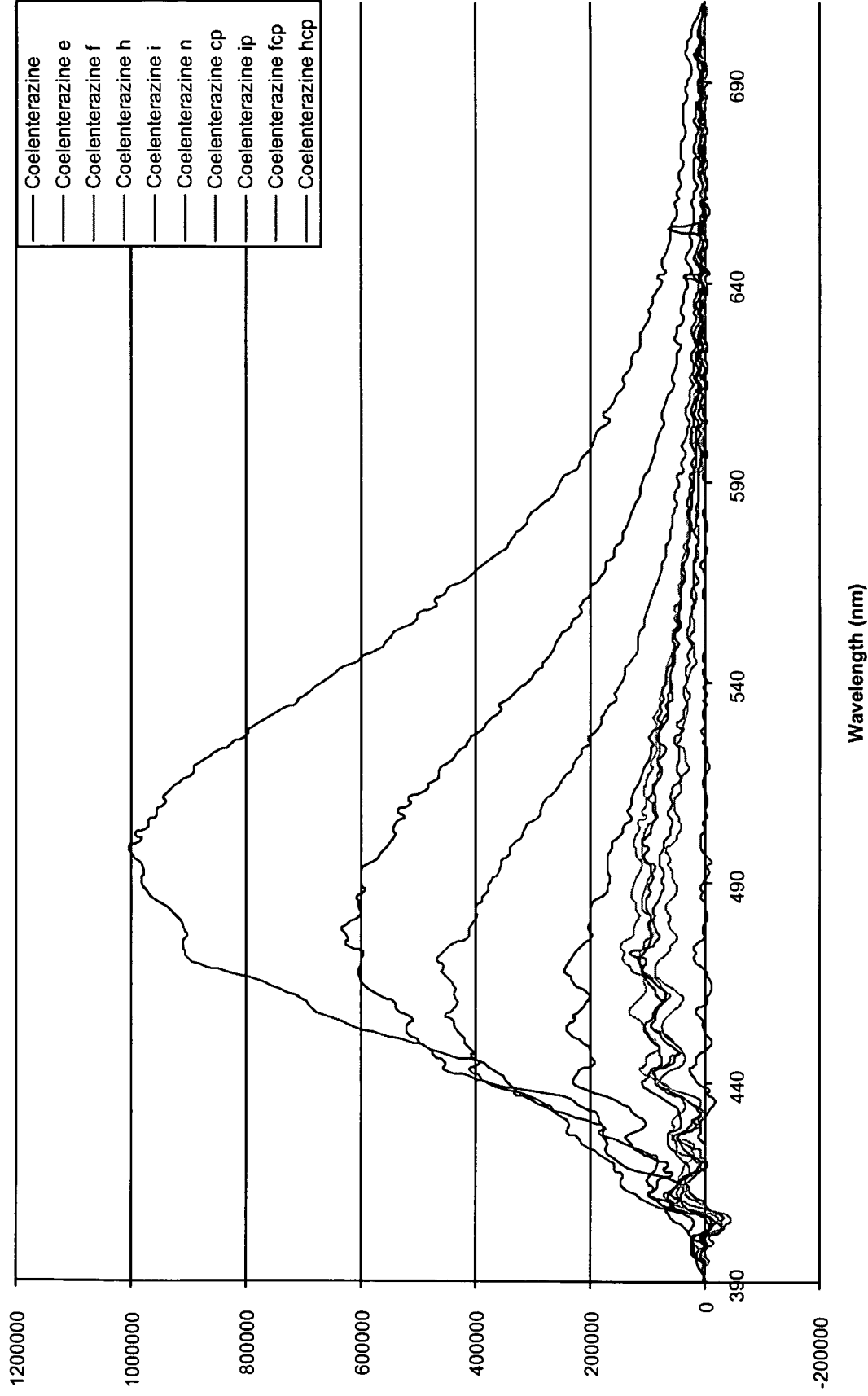


Figure 9
Spectra of 5-Fluoro-L- Tryptophan

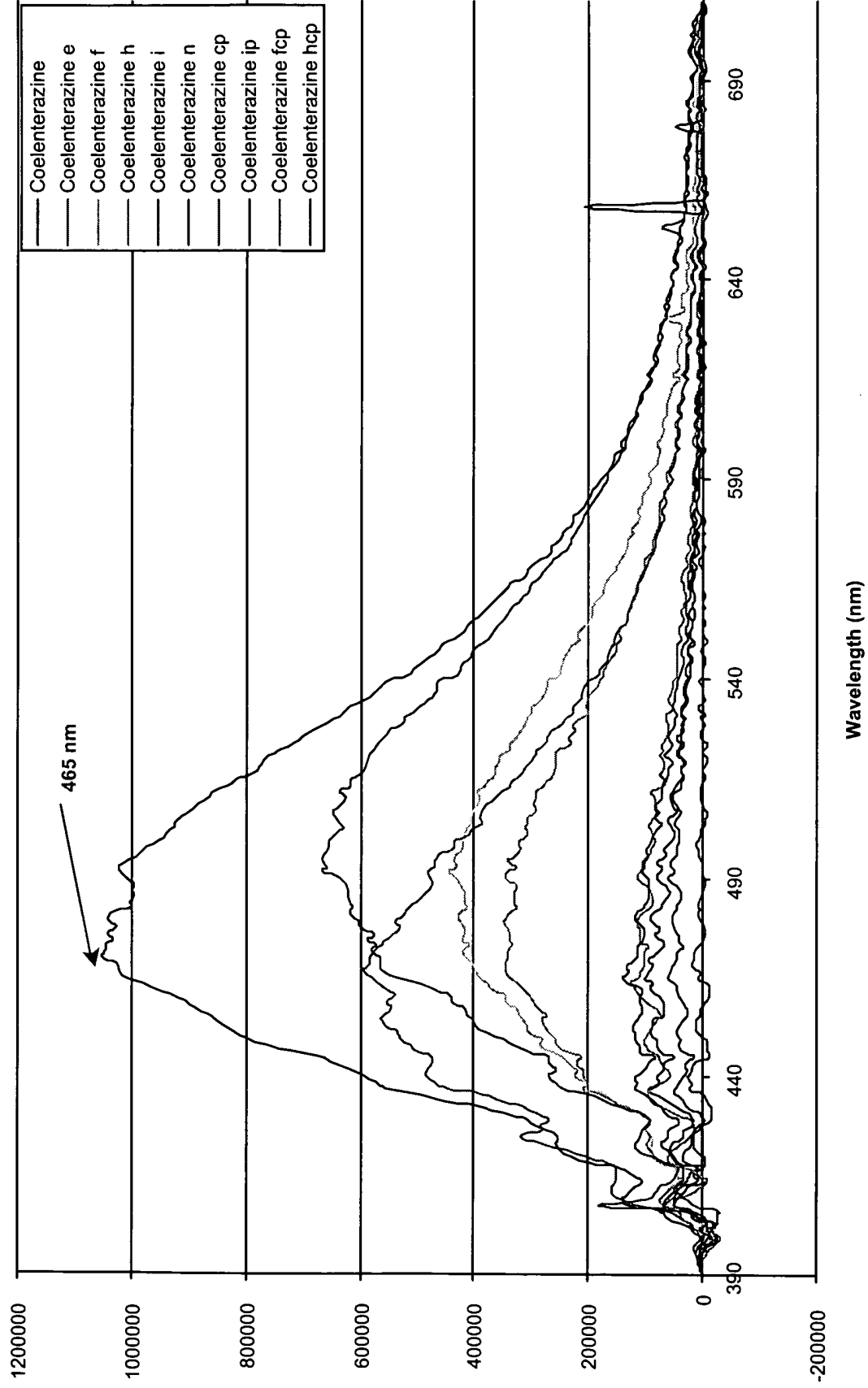


Fig. 10

Energy Transfer Study: Aequorin Mutants

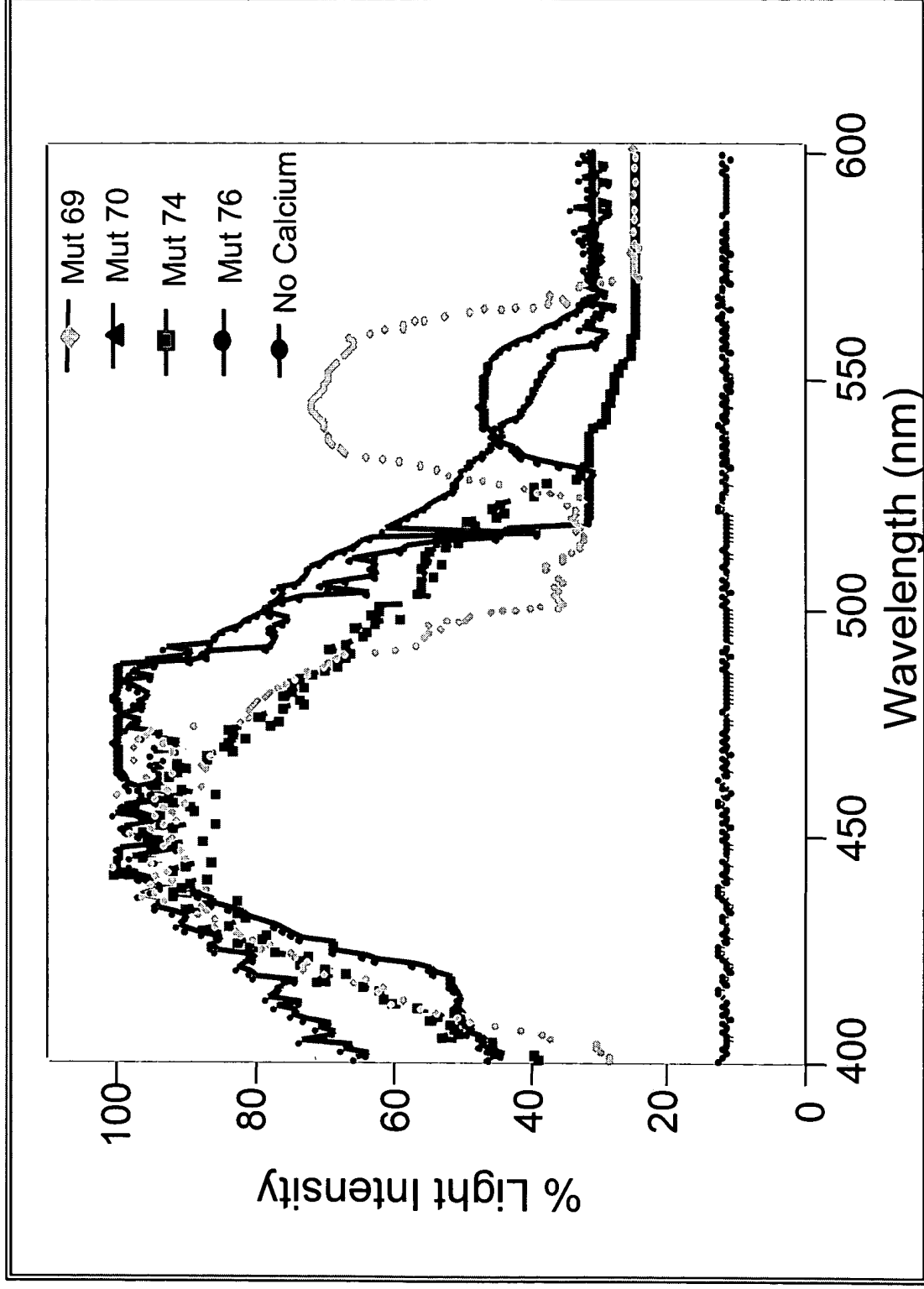


Fig. 11: SEQ ID NO: 1 cDNA encoding wild type apoaeguorin

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1 aatgcaattc atctttgcat caaagaatta catcaaactc
ctagttgatc aactaaattg
61 tctcgacaac aacaagcaaa catgacaagc aaacaatact
cagtcaagct tacatcagac
121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttggag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tgggtgtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gtcacatccg
421 atatgggggtg atgctttgtt tgatatcggt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agattgagag
541 gaaacattca gagtgtgcca tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgctt
gcgaaaagct ctacggtgga
661 gctgtccct aagaagctct acggtggtga tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
841 gaacttacia atcgaaaaag t
```

Fig. 12: SEQ ID NO: 2 amino acid sequence for wild type apoaeguorin

```
VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVKYK SDIVINNPGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDCEETFR
VCDIDESGQL DVDEMTRQHL GFWYTMDPAC EKLYGGAVP
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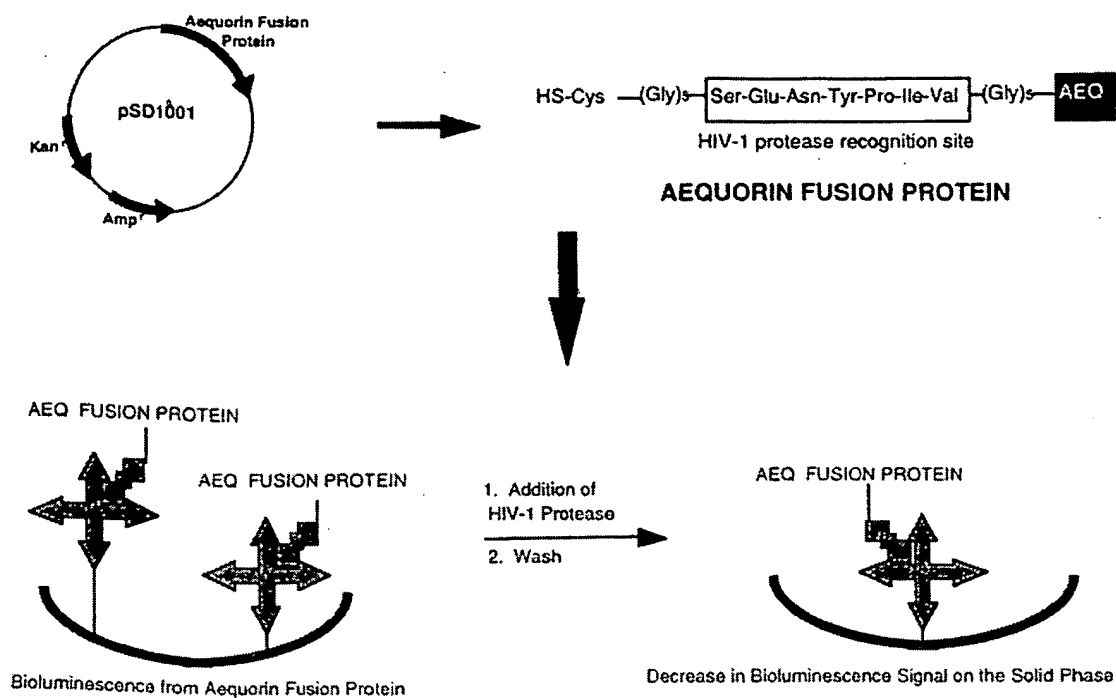
Fig. 13: SEQ ID NO: 3 cDNA encoding Mutant S apoaeguorin

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ctagttgata aactaaattg
61 tctcgacaac aacaagcaaa catgacaagc aaacaatact
cagtcaagct tacatcagac
121 ttcgacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttggag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tgggtgtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa
acgaaccaac gctcatccgt
421 atatgggggtg atgctttggt tgatatcggt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agatagcgag
541 gaaacattca gagtgagcga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgcta
gcgaaaagct ctacggtgga
661 gctgtcccct aagaagctct acggtgggtga tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgtttgt
781 gttgattttt gtaattagga acagattaaa tcgaatgatt
agttgttttt ttaatcaaca
841 gaacttaca atcgaaaaag t
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Fig. 14: SEQ ID NO: 4 amino acid sequence for “Mutant S” apoaeguorin

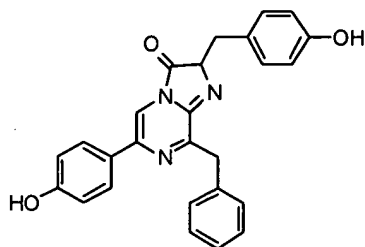
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EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDSEETFR
VSDIDESGQL DVDEMTRQHL GFWYTMDPAS EKLYGGAVP
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Fig. 15

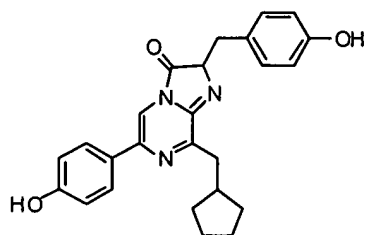


Plasmid construct for the expression of the aequorin fusion protein and schematic representation of the fusion protein showing the HIV-1 protease cleavage site. B represents biotin and NA represents Neutravidin immobilized on the wells.

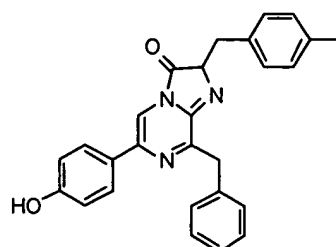
Fig. 16: Coelenterazine Analogs



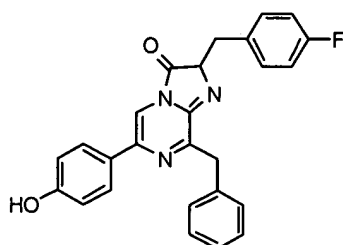
coelenterazine



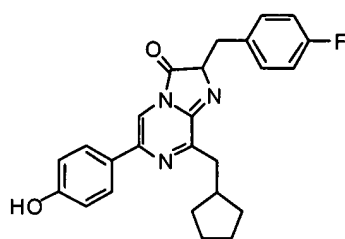
coelenterazine cp



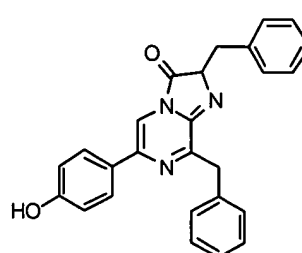
coelenterazine i



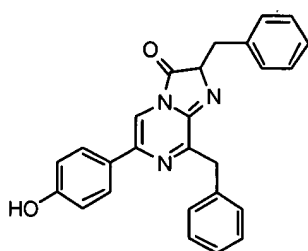
coelenterazine f



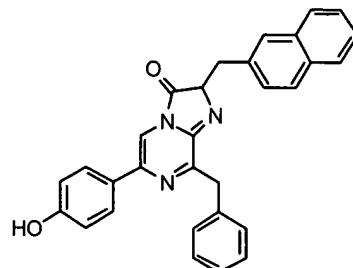
coelenterazine fcp



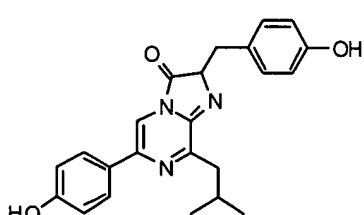
coelenterazine hcp



coelenterazine h



coelenterazine n



coelenterazine ip

Fig. 17. Non-natural amino acids

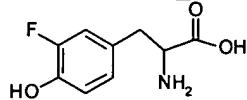
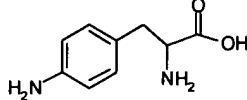
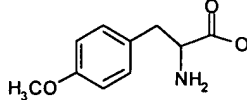
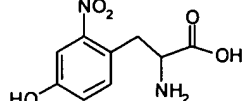
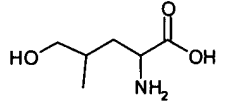
Non-natural Amino Acid Analogue	Representative Example	Effect
Fluoro-analogue	 3-fluoro-L-tyrosine	Altered pK _a and H-bonding strength
Amine-analogue	 <i>p</i> -amino-L-phenylalanine	Altered electrostatic charge and H-bonding strength
Alkyl-analogue	 <i>p</i> -methoxy-L-phenylalanine	Altered H-bonding capability, increased bulkiness
Nitro-analogue	 2-nitro-L-tyrosine	Altered π -character, altered pK _a , altered H-bonding strength, and steric effects
Hydroxyl-analogue	 2-amino-5-hydroxy-4-methylpentanoic acid	Altered pK _a , loss of π - π interactions

Fig. 18 SEQ ID NO: 5, Obelin cDNA sequence

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1  acgatcgaac caaacaactc agctcacagc tactgaacaa
ctcttggttgt gtacaatcaa
61  aatgtcttca aaatacgtag ttaaactcaa gactgacttt
gataatccac gatggatcaa
121 aagacacaag cacatgtttg atttcctcga catcaatgga
aatggaaaaa tcaccctcga
181 tgaaattgtg tccaaggcat ctgatgacat atgtgccaa
ctcgaagcca caccagaaca
241 aacaaaacgc catcaagttt gtgttgaagc tttctttaga
ggatgtggaa tggaatatgg
301 taaagaaatt gccttcccac aattcctcga tggatggaaa
caattggcga cttcagaact
361 caagaaatgg gcaagaaacg aacctactct cattcgtgaa
tggggagatg ctgtctttga
421 tatttttcgac aaagatggaa gtggtacaat cactttggac
gaatggaaa cttatggaaa
481 aatctctggt atctctccat cacaagaaga ttgtgaagcg
acatttcgac attgcgattt
541 ggacaacagt ggtgaccttg atgttgacga gatgacaaga
caacatcttg gattctggta
601 cactttggac ccagaagctg atggtctcta tggcaacgga
gttccctaag ctttttttcg
661 aa
```


Fig. 19. SEQ ID NO: 6, Native Obelin Amino Acid Sequence

MSSKYAVKLK TDFDNPRWIK RHKHMFDFLD INGNKITLD EIVSKASDDI
CAKLEATPEQ TKRHQVCVEA FFRGCGMEYG KEIAFPQFLD GWKQLATSEL
KKWARNEPTL IREWGDAVFD IFDKDGS GTI TLDEWKAYGK ISGISPSQED
CEATFRHC DL DNSGDL DVDE MTRQHLGFWY TLDPEADGLY GNGVP

Fig. 20 Obelin mutants with Coelenterazine analogues.

	Coelenterazine Analogs Emission Max (nm)							
Obelin Mutant	<i>i</i>	<i>ip</i>	<i>h</i>	<i>hcp</i>	<i>cp</i>	<i>fcp</i>	<i>f</i>	<i>ctz</i>
C75S-C51S	521	478	503	478	472	497	503	491
C75S-C67S	505	471	500	471	471	487	504	491
C158S	506	472	497	478	472	475	497	491
C151S	497	471	490	471	471	474	493	491